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Role of glutathione transferases in the improved acclimation to salt stress in salicylic acid-hardened tomato

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ABSTRACT Three weeks old *Solanum lycopersicum* L. cvar. Rio Fuego plants, which grew in hydroponic culture, were pre-treated with 10^{-7} M or 10^{-4} M salicylic acid (SA) and 100 mM NaCl was added to the nutrient solution from the 6th week. The activity of glutathione transferase (GST), glutathione peroxidase (GPOX) and dehydroascorbate reductase (DHAR) were analyzed spectrophotometrically after one week salt stress. All of these activities are connected to GST enzyme family, but the changes were different at the end of the pre-treatment or after the NaCl stress. SA enhanced the GPOX activity in the highest extent by the end of the three-week-period, while in glutathione transferase function there was no significant changes. The salt treatment mostly enhanced these enzyme activities but in the SA-pre-treated plants the GST and GPOX activities were elevated in a higher extent. In contrast to the lower SA concentration, pre-treatment with 10^{-4} M SA maintained the DHAR activities at the control level even in roots. Our results indicate that the increased antioxidant enzyme activities may be the part of the hardening effect of SA. GSTs can participate in the maintenance of the redox state of cells and improving the salt stress tolerance of tomato plants.

Acta Biol Szeged 55(1):67-68 (2011)

KEY WORDSdehydroascorbate reductase
glutathione peroxidase
glutathione transferase activity
salicylic acid
salt stress
tomato

Plant glutathione transferases (GSTs) are a diverse group of multifunctional enzymes that catalyze the conjugation of the reduced form of glutathione (GSH; γ -glu-cys-gly) to diverse electrophilic centres of lipophilic molecules and play important roles in detoxification of cytotoxic endogenous and xenobiotic compounds (Marrs 1996). Some GST isoforms have glutathione peroxidase (GPOX) activity, suggesting that their main function could be the reduction of toxic lipid peroxidation products and the maintenance of membrane integrity. GSTs catalyze alternative GSH-dependent reactions e.g. reduction of dehydroascorbate and also have a role in the metabolism of secondary products such as anthocyanins and cinnamic acid. Arabidopsis contains 55 GST genes which can be divided into 8 classes: phi, tau, theta, zeta, dehydroascorbate reductase (DHAR), lambda, tetrachlorohydroquinone dehalogenase (TCHQD) and microsomal GST (Dixon and Edwards 2010). Some GSTs have important role in hormone metabolism and they can be induced by auxin, ethylene and salicylic acid (SA). SA is a signal molecule and it was shown that SA-generated pre-adaptation responses led to salinity tolerance in tomato (Tari et al. 2002, 2010; Szepesi et al. 2009). Investigation of changes after the 3-week-long adaptation process revealed that tomato plants treated with sub-lethal SA concentrations contained similar H_2O_2 levels than control shoots and roots, but the subsequent salt stress caused lower

H_2O_2 and malondialdehyde levels in the SA-pre-treated plants than in controls after applying 100 mM NaCl for one week (Szepesi et al. 2008; Gémes et al. 2011). In the present work the role of GSTs were investigated in the improved acclimation to salt stress of SA-treated tomatoes.

Materials and Methods

Solanum lycopersicum L. cvar. Rio Fuego plants were grown hydroponically in the greenhouse under $180 \mu\text{mol m}^{-2}\text{s}^{-1}$ light intensity and 12/12 h day/night photoperiod (Szepesi et al. 2008). After 3 weeks control condition we started to pre-adapt the tomato plants with 10^{-7} M or 10^{-4} M SA and 100 mM NaCl was added to the nutrient solution from the 6th week. The GST and GPOX enzyme activities were measured spectrophotometrically using the artificial 1-chloro-2,4-dinitrobenzene (CDNB) and cumene hydroperoxide substrates, respectively as was published earlier (Csiszár et al. 2004). DHAR activity was determined by the method of Edwards and Dixon (2005) after one week of salt treatment. The means \pm SD were calculated from the data of at least three measurements.

Results and Discussion

At the end of the 4-week-long 10^{-7} M SA treatment no significant changes were detectable in the GST activity, the glutathione peroxidase activity was higher and the DHAR activity was lower than in controls. The GPOX and DHAR activities were enhanced in higher extent after the 10^{-4} M SA

Accepted July 11, 2011

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pre-treatment, especially in the roots. The activities of these enzymes were mostly elevated due to salt stress but the GST and GPOX activities were more induced in SA pre-treated plants after applying 100 mM NaCl. These results suggest that the GSTs may have important function in the stress response due to their role in detoxification of toxic stress metabolites (e.g. lipid peroxides), so preventing the membrane damages. The DHAR activity of stressed plant remained about the control level in roots of 10^{-4} M SA pre-treated plants in contrast to the decreased activity of 10^{-7} M SA pre-treated tomatoes, which is in a good correlation with the more effective hardening of the 10^{-4} M SA treatments. DHARs are unable to catalyse typical GST conjugating reactions using GSH because they have an active site cysteine instead of serine/tyrosine, and are more likely to catalyse redox reactions (Dixon et al. 2010). DHARs reduce dehydroascorbate to ascorbate while oxidise GSH to glutathione disulfide and they have a role in maintaining the reduced ascorbate pool (Dixon et al. 2002). Consequently the GST enzymes can participate in the maintenance of the redox state, metabolism, function and structure of cells (Dixon et al. 2010). Our results indicate that the increased GST enzyme activities with diverse functions may be the part of the hardening effect of SA and improve the salt stress tolerance of tomato plants in a complex way.

Acknowledgements

This work was supported by the Hungarian National Scientific Research Foundation (OTKA K76854). A part of this study was presented on the 10th Congress of the Hungarian Society for Plant Biology, August 31 - September 2, 2011, Szeged, Hungary.

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